Ecophysiology of seed dormancy and the control of germination in early spring-flowering Galanthus nivalis and Narcissus pseudonarcissus (Amaryllidaceae)

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Ecophysiology of seed dormancy and the control of germination in early spring-flowering *Galanthus nivalis* and *Narcissus pseudonarcissus* (Amaryllidaceae)

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Running title: Seed germination in *Galanthus* and *Narcissus*

Seed dormancy induction and alleviation in the winter-flowering moist temperate woodland species *Galanthus nivalis* and *Narcissus pseudonarcissus* are complex and poorly understood. Temperature, light and desiccation were investigated to elucidate their role in the germination ecophysiology of these species. Outdoor and laboratory experiments simulating different seasonal temperatures, seasonal durations, and temperature fluctuations; the presence of light during different seasons; and intermittent drying (during the summer period) over several 'years' investigated the importance of these factors in germination. Warm summer-like temperatures (20°C) were necessary for germination at subsequent cooler autumn-like temperatures (greatest at 15°C in *G. nivalis* and 10°C in *N. pseudonarcissus*). As the warm temperature duration increased so did germination at subsequent cooler temperatures; further germination occurred in subsequent 'years' at cooler temperatures following a second, and also third, warm period. Germination was significantly greater in darkness, particularly in *G. nivalis.* Dormancy increased with seed maturation period in *G. nivalis*, because seeds extracted from green capsules germinated more readily than those from yellow. Desiccation increased dormancy in an increasing proportion of *N. pseudonarcissus* seeds the later they were dried in 'summer'. Seed viability was only slightly reduced by desiccation in *N. pseudonarcissus* but was poor and variable in *G. nivalis*. Shoot formation occurred both at the temperature at which germination was greatest and also if 5°C cooler. In summary, continuous hydration of seeds of both species during warm summer-like temperatures results in the gradual release of seed dormancy; thereafter, darkness and cooler temperatures promote germination. Cold temperatures, increased seed maturity (*G. nivalis*), and desiccation (*N. pseudonarcissus*) increase dormancy while light inhibits germination.

ADDITIONAL KEYWORDS: climate – darkness – desiccation – light – seed viability – temperate woodland geophytes – temperature requirement.

INTRODUCTION

Many temperate plant species produce seeds that are dormant when dispersed (Baskin & Baskin, 2001). Seed dormancy prevents germination in conditions appropriate for germination but unsuitable for seedling establishment (Vleeshouwers, Bouwmeester & Karssen, 1995), ensuring that seeds germinate at the best possible time to maximise the chances of plants establishing and reproducing (Harper, 1977).

Seeds from temperate plant communities that germinate in spring often require a period of cold stratification to prevent germination during a period of mild temperatures in the winter if dispersed in autumn, such as *Silene elisabethae* Jan (Mondoni *et al.*, 2009a), or warm followed by cold stratification if shed in spring, as in *Scilla bifolia* L. (Vandelook & van Assche, 2008). Occasionally seeds require a cold, warm, cold stratification sequence, for example *Cardiocrinum cordatum* (Thunb.) Makino var. *glehnii* (F.Schmidt) H.Hara (Kondo *et al*., 2006), before germination will occur. Autumn germination also occurs in temperate regions; in species such as *Hyacinthoides non-scripta* L. (Thompson & Cox, 1978; Vandelook & van Assche, 2008) and *Anemone nemorosa* L. (Mondoni *et al.*, 2008), a period of warm stratification is often required prior to germination in the autumn.

The genus *Narcissus* L. originated in Spain, from where species have spread in all directions (Blanchard, 1990; Meerow *et al.*, 2006). *Narcissus pseudonarcissus* L., the wild daffodil or Lent lily, is indigenous to southern Europe and the western Mediterranean region, was possibly introduced to the United Kingdom by Roman settlers in the fourth and fifth centuries (Church, 1908) and has naturalised in many places (Blanchard, 1990). The ancestor of the

genus *Galanthus* L. is likely to have originated in the Caucasus or Mediterranean Europe (Meerow *et al*., 2006). *Galanthus nivalis* L. (the common snowdrop) is native to western, central and southern Europe, and although not indigenous to the British Isles, has naturalised extensively from escaped garden plants (Church, 1908; Bishop, Davis & Grimshaw, 2001). Plants of *G. nivalis* plants naturally inhabit humid places that may be flooded for short periods (Bishop *et al.*, 2001). In contrast, *N. pseudonarcissus* plants are found growing in a wider variety of habitats: in meadows and open woodlands or along river banks, although they grow best in damp areas (Church, 1908; Caldwell & Wallace, 1955).

Narcissus and *Galanthus* species are economically important as ornamentals in the horticultural industry (Bishop *et al*., 2001; Nuñez *et al.*, 2003) and species of *Galanthus*, in particular, have attracted attention for their potential in treating Alzheimer's disease (Heinrich & Teoh, 2004). Survival of populations of Amaryllidaceae plants in the wild is becoming increasingly threatened by bulb collection from the natural habitat for the horticultural bulb trade (Budnikov & Kricsfalusy, 1994; Davis, 1999), and this, combined with the threat of habitat loss, has resulted in several *Narcissus* and *Galanthus* species being listed on the IUCN Red List of Threatened Species, including *G. nivalis* as near-threatened. (Crook & Davis, 2013; IUCN, 2014).

Promoting seed germination of these species has been problematic, with most knowledge indicating the difficulties. For example, for *Narcissus* seed germination there is a long dormancy period (*N. pseudonarcissus*: Caldwell & Wallace, 1955), cold stratification fails to break dormancy (*N. bulbicodium* L.: Thompson, 1977), and seeds require summer temperatures (Thompson, 1977) for germination in the autumn (Caldwell & Wallace, 1955;

Thompson, 1977). More recent papers have, however, reported on aspects of seed dormancy and germination in several *Narcissus* species (Copete *et al.*, 2011; Marquis & Draper, 2012; Herranz, Copete & Ferrandis, 2013a, b), and the effects of temperature on seed development, embryo growth, seed germination, seedling development and seedling emergence in *N. pseudonarcissus* have been investigated (Vandelook & van Assche, 2008; Newton, Hay & Ellis, 2013), although the latter research did not extend to examining the effect of light conditions on seed dormancy. No studies to our knowledge have examined the environmental and temporal regulation of seed germination in any species from the genus *Galanthus*. Moreover, the effects of seed maturity on dormancy and drying during the summer period on seed viability and subsequent germination in these genera are unknown.

The overarching hypothesis of this study is that the apparent complexity of promoting the *ex-situ* germination of seeds of early spring-flowering temperate Amaryllidaceae species *G. nivalis* and *N. pseudonarcissus* is a consequence of their ecological niche and evolutionary history. The main objective of this study was to elucidate and compare the germination ecophysiology of these species in the context of temperature, light and desiccation during stratification, with specific aims to investigate: (*a*) whether the period that seeds experience warm summer temperatures affects germination in the subsequent autumn; (*b*) whether germination is affected by alternating temperature; (*c*) the effect of light in different seasons on germination; (*d*) the effect of desiccation during the summer period on germination and viability; (*e*) whether seed maturity affected dormancy; and (*f*) the effect of temperature on shoot formation.

MATERIALS AND METHODS

STUDY SITES AND SEED COLLECTION

Seeds were collected from different populations over several years; seed lots are uniquely identified by the species, locality and year of collection (Table 1). *Narcissus pseudonarcissus* seeds were collected from wild populations in the Loder Valley Nature Reserve, West Sussex (51°03’29’’N, 00°05’33’’W) and Forest Edge Farm near Ringwood, South Hampshire (50°51’55’’N, 01°45’35’’W). As suitable *N. pseudonarcissus* populations for this study were small, seed was sourced from two different localities to ensure that seed removal was within recommended guidelines so as to not threaten the survival of sampled populations (Way, 2003). *Galanthus nivalis* seeds were collected from an introduced population at Wakehurst Place, West Sussex (Newton *et al.*, 2013). A minimum of 30 individual plants per population were sampled in both species. Capsules of *G. nivalis* were collected as soon as the flower stalk started to disintegrate. As capsules were predominantly green on collection, they were placed in a monolayer on blotting paper in a plastic tray at 75% RH and 15°C for 1 – 20 d, simulating the natural environment for continued ripening *ex planta*, until capsule dehiscence and seed release (Newton *et al.*, 2013). Capsules of *N. pseudonarcissus* that were brown and starting to split were also placed at 75% RH and 15°C (reflecting the mean ambient conditions at the study site at the time) for 1 – 5 d. Seed samples were sorted by eye to ensure that only firm, fully-formed seeds were selected for study. All germination tests commenced within a week of capsule dehiscence.

TEMPERATURE AND LIGHT MEASUREMENTS IN THE FIELD

Mean weighted daily temperature from May 2011 for one year was calculated for the outdoor pot experiment from the maximum and minimum temperatures (obtained from the

Horticulture Section at Wakehurst Place) using the following equation (where T = temperature):

Mean weighted daily T = [(maximum T × light hours) + (minimum T × dark hours)] / 24

Two Tiny Tag PT loggers (Gemini Data Loggers (UK) Ltd., Chichester, UK), one positioned approximately 100 mm above the ground within the Loder Valley Nature Reserve, and the second heat-sealed in a foil bag and buried 5 mm below the soil surface, recorded temperature every 20 minutes for one year from October 2008 for temperature amplitude determination both above and below ground.

The photon flux density of light in the range of 400 – 700 nm was determined on three separate occasions for different microsites in the Loder Valley Nature Reserve using a Q.101 quantum radiometer / photometer (Macam Photometrics Ltd., Livingston, UK). Light measurements were taken at the beginning of autumn in September 2010 at four different microsites: bare ground (full sunlight), woodland (dappled shade), grass cover (shaded) and under leaf litter (shaded) in both sunny and cloudy conditions.

OUTDOOR EXPERIMENTS

Freshly-collected seeds ($n = 8 \times 25$ per species) of *G. nivalis* Wakehurst 2011 and *N. pseudonarcissus* Loder 2011 seed lots were sown 40 mm deep in compost in each of 8 plastic pots (200 mm diameter) and placed outside in the Wakehurst Place plant nursery. One pot per species was randomly selected at monthly intervals and any emergent seedlings recorded.

Thereafter, the soil was removed and sieved and the seeds recovered and examined for evidence of germination (radicle emergence > 2 mm) and shoot formation (first leaf > 5 mm and distinct from the cotyledon limb).

LABORATORY EXPERIMENTS

Sample sizes throughout the investigations were necessarily small to avoid damaging the sampled plant populations, with the different seed samples (locations and/or years) providing compensation for this. Treatment combinations in *N. pseudonarcissus* comprised samples of 50 seeds per germination test. In *G. nivalis* they consisted of two replicates of 50 seeds in all experiments, except for the partial factorial experiment investigating the effect of temperature and light on germination (50 seeds per treatment), the seed maturity study (25 seeds per treatment) and the shoot formation study (38 seeds per treatment). Seeds were sown on 1% distilled water agar held in 10 mm diameter Nunc multiwell dishes (Scientific Laboratory Supplies Ltd., Nottingham, UK), which were used to avoid fungal spread between seeds during the long test periods (Newton *et al.*, 2013). Dishes were placed in clear plastic bags (Fisher Scientific UK Ltd., Loughborough, UK) in cooled incubators (LMS Ltd., Sevenoaks, UK) at different temperatures with lateral illumination provided by 30 W cool white fluorescent lights.

Unless otherwise stated, *N. pseudonarcissus* germination tests were conducted in the light which was provided for 8 h d^{-1} (during the warm phase where alternating temperatures were used); *G. nivalis* germination tests were conducted in the dark by double-wrapping dishes in aluminium foil prior to incubation. Germination was assessed at regular intervals under a flow hood (Capitarhood, Bigneat Ltd., Waterlooville, UK) or, for tests in the dark, in a dark

room under a dim safe, green light comprising three 15 – 20 W cool white fluorescent tubes covered by three layers of no. 39 (primary green) Cinemoid (Probert & Smith, 1986).

The criterion for germination was radicle emergence (> 2 mm). Seeds were transferred to fresh agar at least every 84 d. Fungal growth, when present, was gently removed from seeds using fine dissecting forceps (Agar Scientific Ltd., Stansted, UK). At the end of each test, seeds that had not germinated were dissected to determine whether they were still firm (and so assumed viable).

Temperature regimes. Germination tests were move-along experimental designs, modified from Baskin and Baskin (2003). Alternating (8/16 h) temperatures of 25/10°C for summer, 15/5°C for autumn and spring and 10/0°C for winter were provided for initial *N. pseudonarcissus* experiments. Constant temperatures were, however, chosen for subsequent experiments: 20°C for summer, 10°C for autumn and spring, and 5°C for winter for *N. pseudonarcissus*; 20°C for summer, 15°C for autumn and spring, and 10°C for winter for *G. nivalis*. The temperature regimes for *N. pseudonarcissus* were selected to simulate natural, realistic temperatures for the region, averaged for each season; however, as germination was poor in *G. nivalis* at these temperatures and initial experiments suggested the optimum temperature for germination was warmer, autumn and spring temperatures of 15°C were chosen to provide greater differences in germination between treatments in comparative studies. Deviation from the above temperatures in specific tests investigated the effect of different seasonal temperatures and is indicated where used. Unless otherwise stated, the temperature regimes detailed above were used in experiments, and germination tests began with the summer season, with an 84 d (12 week) seasonal period (providing a 48-week 'year').

Effect of seasonal duration on seed germination. The duration of the first summer period was reduced from the standard 84 d to 0, 14, 28, 42, 56 or 70 d, or extended to 98 d in *N. pseudonarcissus* Loder 2006, and reduced to 0, 28 and 56, or extended to 112, 140 or 168 d in *N. pseudonarcissus* Ringwood 2007 and *G. nivalis* Wakehurst 2008 seed lots. If the duration of the first summer was 0 d, germination tests were put directly into the first autumn environment. All seasons following the first summer were the standard 84 d in duration. In a subsequent experiment, the duration of all seasons were 84, 56 or 28 d (all starting in the summer) in *N. pseudonarcissus* Ringwood 2006, *N. pseudonarcissus* Loder and Ringwood 2008, and *G. nivalis* Wakehurst 2008 seed lots. Investigations with the 28 d cycle finished at the end of the fifth autumn {[(28 + 28 + 28 + 28) × 4 + 28 + 28] = 504 d}, the 56 d cycle at the end of the third autumn {[(56 + 56 + 56 + 56) × 2 + 56 + 56] = 560 d}, and 84 d cycle at the end of the second autumn {[(84 + 84 + 84 + 84) × 1 + 84 + 84] = 504 d}. Temperature regimes for investigations carried out on *N. pseudonarcissus* Loder 2006 and Ringwood 2006 seed lots were alternating, while constant temperature regimes were provided for *N. pseudonarcissus* Ringwood 2007, Ringwood 2008, Loder 2008 and *G. nivalis* Wakehurst 2008. All *N. pseudonarcissus* treatments were in the light and *G. nivalis* in the dark.

Effect of constant and alternating temperatures on seed germination. Alternating temperatures, with either a 10 or 15°C diurnal range, were selected to provide the same daily arithmetic mean as equivalent constant temperatures of 20°C [25/15°C (12/12 h) and 30/15°C (8/16 h)], 15°C [20/10°C (12/12 h) and 25/10°C (8/16 h)], 10°C [15/5°C (12/12 h) and 20/5°C (8/16 h)], and 5°C [10/0°C (12/12 h) and 15/0°C (8/16 h)]. Mean seasonal temperatures (summer-autumn-winter-spring) were 15-10-5-10°C in *N. pseudonarcissus* Loder 2007, 20-10-5-10 and 20-15-10-15°C in *N. pseudonarcissus* Loder 2008, and 20-15-

10-15°C in *G. nivalis* Wakehurst 2008. All treatments were in the dark, with the exception of the *N. pseudonarcissus* Loder 2007 seed lot, which was in the light.

Effect of light and darkness on seed germination. Seeds from *N. pseudonarcissus* Loder 2007, Ringwood 2008 and *G. nivalis* Wakehurst 2008 seed lots were subjected to various regimes, including: (*a*) light summer, light autumn, light winter, light spring (LLLL); (*b*) dark summer, light autumn, light winter, light spring (DLLL); (*c*) light summer, dark autumn, dark winter, dark spring (LDDD); (*d*) dark summer, dark autumn, dark winter, dark spring, assessed under a safe, green light (DDDD-D); and (*e*) dark summer, dark autumn, dark winter, dark spring, assessed under laboratory light (DDDD-L); with alternating (*N. pseudonarcissus* Loder 2007) or constant (*N. pseudonarcissus* Ringwood 2008 and *G. nivalis* Wakehurst 2008) temperatures. Temperature regimes, selected to simulate natural temperatures for the region, are described earlier.

Additional constant temperature experiments (N = 18 treatments per species; n = 50 seeds per treatment) with *N. pseudonarcissus* Loder 2008 and *G. nivalis* Wakehurst 2007 populations, with light either present or absent in all seasons, investigated the relative importance of light and temperature to germination. In *N. pseudonarcissus*, an average summer of 20°C was combined factorially with: a cool (5°C), average (10°C) or warm (15°C) autumn; a cool (0°C), average (5°C) or warm (10°C) winter; and an average spring (10°C). In *G. nivalis*, the factorial combinations were: a cool (15°C), average (20°C) or warm (25°C) summer with a warm autumn (15°C), warm winter (10°C) and warm spring (15°C); a warm autumn (15°C), average winter (5°C) and warm spring (15°C); or an average autumn (10°C), average winter (5°C) and average spring (10°C). Seeds of *N. pseudonarcissus* were placed directly into

summer temperatures; *G. nivalis* seeds, however, were subjected to an initial 28 d spring prior to the first summer. Seeds germinated in the light were assessed in the light; seeds germinated in the dark were assessed under a safe, green light.

Effect of desiccation on seed germination. Seeds of *N. pseudonarcissus* Loder 2006 and Loder 2008 and *G. nivalis* Wakehurst 2008 were dried for 14 d at 15% RH and 15°C at different times during the summer and autumn. Drying treatments comprised: a control (not dried); dried once at 0 d (beginning of summer), 28 d (1/3 through summer), 56 d (2/3 through summer), 84 d (end of summer), 91 d (7 d into autumn) and 105 d (21 d into autumn) after the germination test began; or two (42, 84 d), three (24, 56, 84 d) or four times (21, 42, 63, 84 d) during the first summer period. Seeds were removed from agar for drying and returned to fresh agar at the same point in the seasonal move-along sequence. The summer period resumed at the point of removal from agar so that all treatments received an imbibed 84 d summer and 84 d autumn period. Alternating temperature regimes were provided for the *N. pseudonarcissus* Ringwood 2006 seed lot and constant temperatures for the *N. pseudonarcissus* Ringwood 2008 and *G. nivalis* Wakehurst 2008 seed lots; all *N. pseudonarcissus* tests were in the light and all *G. nivalis* tests in the dark. Temperature regimes, selected to simulate natural temperatures for the region, are described earlier.

Effect of seed maturity on seed germination. Seeds extracted from green or yellow capsules, collected at the same time from the *G. nivalis* Wakehurst 2007 population (Table 1), were placed into constant temperature regimes (N = 18 treatments, n = 25 seeds per treatment) combined factorially as follows: a cool (15°C), average (20°C) or warm (25°C) summer with a warm autumn (15°C), warm winter (10°C) and warm spring (15°C); a warm autumn

(15°C), average winter (5°C) and warm spring (15°C); or an average autumn (10°C), average winter (5°C) and average spring (10°C). All treatments were in darkness and assessed for germination under a safe, green light.

Effect of temperature on shoot formation. Seeds of *N. pseudonarcissus* Loder 2009 and *G. nivalis* Wakehurst 2009 seed lots were placed on agar in the dark at 20°C for 84 d and then moved to 10 and 15°C, respectively. Seeds were checked regularly for germination; as soon as the radicle was > 5 mm in length, germinated seeds (n = 50 per treatment for *N. pseudonarcissus* and n = 38 for *G. nivalis*) were transferred to agar in polyethylene boxes (170 × 115 × 60 mm) and placed at either 10 or 5°C (*N. pseudonarcissus*) or at 15 or 10°C (*G. nivalis*) in the light. Shoot development was scored as complete when the first leaf was observed to be > 5 mm in length and distinctly separate from the cotyledon limb.

DATA ANALYSIS

All analyses were carried out in Genstat for Windows Eleventh Edition (VSN International Ltd., Hemel Hempstead, UK). Seed germination in variable duration summer experiments was analysed by probit analysis. Logistic regression analysis was used to test for significant differences between treatments. Unless otherwise indicated, errors presented are standard errors (s.e.).

RESULTS

TEMPERATURE AND LIGHT MEASUREMENTS IN THE FIELD

The photon flux density of photosynthetically active radiation, received in the four different microsites in the Loder Valley Nature Reserve, ranged from 2000 µmol m^{-2} s^{-1} in full sunlight

on a bright day to 0.1 μmol m^{-2} s^{-1} under leaf litter on a cloudy day, with incubator illumination similar to light levels on the soil surface of dappled shady woodland or shaded grass (Table 2). Over a year, the mean temperature amplitude (the difference between the maximum and minimum temperature over a 24 h period, starting at midnight) in Loder Valley Nature Reserve in air was 8.8 ± 0.21°C and 5 mm below the soil surface was 2.8 ± 0.09°C.

OUTDOOR EXPERIMENTS

Radicle emergence (germination) was first observed in late September in *G. nivalis* (Fig. 1B) and early October in *N. pseudonarcissus* (Fig. 1A) following a steady decrease in the mean maximum and weighted temperature over the preceding eight weeks from 23.8 to 19.2°C and 19.5 to 15.9°C, respectively (Fig. 1C). The majority (> 75%) of seeds of both species had germinated by the end of October, when the mean weighted temperature had dropped to 10.3°C (Fig. 1). Shoot formation followed one (*G. nivalis*) to two (*N. pseudonarcissus*) months after radicle emergence. Radicle and shoot elongation continued slowly over the winter in both species, with shoot emergence from the soil in February (Fig. 1A, B) at the end of the cold winter period (Fig. 1C).

LABORATORY EXPERIMENTS

Effect of seasonal duration on seed germination. In both *N. pseudonarcissus* and *G. nivalis*, an increase in the duration of moist warm stratification (20°C) simulating summer increased germination in the subsequent cooler autumn (Fig. 2). This response was normally distributed, with 50% of the maximum germination achieved after 76 d of warm stratification

in *G. nivalis* (Fig. 2C) and 60 d in *N. pseudonarcissus* (Fig. 2B). Greater germination of seeds in the second autumn occurred in treatments with summer periods of shorter duration (Fig. 2A-C). Further, limited germination occurred in several treatments in the third autumn (Fig. 2B), resulting in similar final total germination in all treatments within species (Fig. 2), with the exception of the 140 d treatment in *G. nivalis* (Fig. 2C), whose seed viability was negatively affected by high levels of fungal contamination.

Reducing the duration of all seasons significantly reduced seed germination at suitable temperatures ($P < 0.001$, logistic regression; Fig. 3) in both species: at the end of the first autumn there was 1 ± 1.0% (s.e.) germination for the 28 d, 18 ± 10.3% for the 56 d and 45 ± 9.2% for the 84 d season treatments ($n = 4$, averaged across all seed lots). This pattern of decreased germination in shorter duration seasons was maintained in all collections, with the exception of *N. pseudonarcissus* Ringwood 2008, in which germination at the end of the first winter in 56 and 84 d season treatments was similar (Fig. 3). In *N. pseudonarcissus*, germination occurred predominantly when the temperature dropped to 15/5°C (91% of germination in *N. pseudonarcissus* 2006 occurred in autumn) or 10°C (91% in *N. pseudonarcissus* Loder 2008 autumn and 73% in *N. pseudonarcissus* Ringwood 2008 winter) while in *G. nivalis* germination occurred mostly when the temperature dropped to 15°C (74% in *G. nivalis* 2008 autumn).

Effect of constant and alternating temperatures on seed germination. Germination was greatest at constant temperatures compared with alternating temperatures, in the light in *N. pseudonarcissus* (*N. pseudonarcissus* 2007: 88% at constant temperatures compared with 70% at alternating temperatures) and dark in *G. nivalis* (*G. nivalis* 2008: 42% at constant

temperatures compared with 17 and 11% at alternating temperatures) (Fig. 4). In both species, germination occurred most rapidly at constant temperatures with germination rate decreasing as the diurnal temperature range increased. For example, at the end of the first autumn, germination at constant, alternating 10°C and alternating 15°C, was 60, 18 and 2% [*N. pseudonarcissus* 2007 (L): 10°C autumn]; 94, 90 and 72% [*N. pseudonarcissus* 2007 (D): 10°C autumn]; 80, 16 and 2% [*N. pseudonarcissus* 2007 (D): 15°C autumn] and 28, 4 and 1% [*G. nivalis* 2008 (D): 15°C autumn], respectively (Fig. 4). A logistic regression showed germination at alternating temperatures was significantly different from constant temperatures at the end of the first autumn ($P < 0.001$). Germination in all *N. pseudonarcissus* treatments in the dark was complete by the end of the first winter (≥ 90%), irrespective of temperature fluctuation.

Effect of light and darkness on seed germination. Germination in both species was significantly greater in darkness than light ($P < 0.001$ in both, logistic regression), with most germination taking place in the autumn over successive seasons (Fig. 5). In dark treatments, seed germination was virtually complete in *N. pseudonarcissus* by the end of the first autumn (LDDD and DDDD treatments, Fig. 5A). Darkness during summer (DLLL and DDDD-D *cf.* LLLL and LDDD) did not improve autumn germination ($P = 0.701$, logistic regression) and a brief weekly exposure to light when seeds in the dark were assessed for germination (DDDD-L) had a negligible effect on *N. pseudonarcissus* germination compared with the same treatment scored in the dark ($P = 0.465$, logistic regression). In contrast, darkness in summer slightly increased (DLLL and DDDD-D *cf.* LLLL and LDDD, $P = 0.002$, logistic regression), while a brief weekly exposure to light slightly reduced (DDDD-L *cf.* DDDD-D, $P = 0.006$, logistic regression), final germination in *G. nivalis* (Fig. 5A).

At the end of the first autumn, mean germination of *N. pseudonarcissus* seeds in the dark (n = 9) was 78 ± 4.3% (s.e.); 51 ± 3.8% greater in the dark than in the light (Fig. 5B). In subsequent autumn seasons, (where autumn was 5° and 10°C), further germination occurred in all light and some dark treatments, reducing the difference between light and dark in final germination to 27 ± 5.6% (n = 6, Fig. 5B). When autumn was 15°C, most germination occurred at this temperature in dark treatments while a greater proportion of germination in light treatments was delayed until the winter season when the temperature was 10°C (Fig. 5B).

A similar, but more pronounced, pattern of greater germination in dark treatments was evident in *G. nivalis*: germination in successive, predominantly autumn, seasons resulted in a mean germination at the end of the third autumn of 69 ± 6.4% (n = 9); 56 ± 6.8% greater than germination in the light: 12 ± 5.4% (Fig. 5C). Germination was reduced in dark treatments where autumn was 10°C compared with that at 15°C (Fig. 5C). Logistic regression analysis showed that temperature and light significantly affected seed germination in both species (P < 0.001).

Effect of desiccation on seed germination. In *N. pseudonarcissus*, drying seeds in summer or at the beginning of autumn reduced germination in the autumn, compared with freshly-collected seeds (Fig. 6A, B). The later that drying occurred in the summer period (reducing the duration in summer following drying), the fewer seeds germinated in the subsequent autumn (Fig. 6A, B), which was significant (P < 0.001, logistic regression). Drying in

summer slightly reduced seed viability (P = 0.026, logistic regression) by a maximum of 14% in 2006 (Fig. 6A) and 10% in 2008 (Fig. 6B); when drying in autumn was included in the analysis, this was more pronounced (P < 0.001, logistic regression). Seeds dried more than once during the summer period behaved similarly to seeds dried at the end of the summer period (day 84): germination patterns were similar and viability did not differ in these treatments (P = 0.536, logistic regression). Germination in all *G. nivalis* treatments was poor (< 50%) and quite variable, although a similar trend to that in *N. pseudonarcissus* of reduced germination in the first autumn following drying, and also drying later in the summer period, was evident (Fig. 6C). Prolific fungal infection, in spite of regular inspection and agar changes, reduced *G. nivalis* viability and consequently germination.

Effect of seed maturity on seed germination. Germination in the first autumn in seed extracted from green capsules was equal to or greater than germination of seed from yellow capsules in all but one treatment (20-10-5-10°C), resulting in 52 ± 12.5% germination in seeds from green capsules compared with 33 ± 9.0% in yellow capsules (n = 9, Fig. 7). This pattern was largely maintained in subsequent seasons, with final germination in seeds from green capsules greater than that from yellow capsules in again, all but one treatment (15-15-5-15°C) (Fig. 7). Overall, germination was greater in seeds extracted from green, compared with yellow, capsules (P = 0.002, logistic regression).

Effect of temperature on seed germination. Temperature affected both the rate and final germination in both species. Following a warm period of 20°C, germination was greatest at 10°C in *N. pseudonarcissus* (n = 3) in the light (47 ± 7.5%) compared with 5°C (10 ± 3.1%) and 15°C (25 ± 1.3%); and similarly so in the dark, with 93 ± 0.7% at 10°C, compared with

66 ± 6.4% (5°C) and 75 ± 0.7% (15°C) (Fig. 5B). In *G. nivalis*, following a warm period of 20°C, greatest germination (82 ± 3.3%, *n* = 2) occurred at the warmer temperature of 15°C in the dark, compared with only 20% (*n* = 1) at 10°C (Fig. 5C). The same pattern with greatest germination occurring at 10°C in *N. pseudonarcissus* and 15°C in *G. nivalis* was evident irrespective of seasonal duration (Fig. 3) and also seed maturity: following a warm period of 20°C, germination was 82 ± 7.7% (*n* = 4) at 15°C compared with 20 ± 4.0% (*n* = 2) at 10°C (green and yellow capsule data combined, Fig. 7). In the field, *G. nivalis* seed germination commenced at temperatures of around 16°C, somewhat earlier than *N. pseudonarcissus* (Fig. 1).

Following a warm period of 20°C, 37 ± 8.5% (*n* = 3) greater germination occurred in *N. pseudonarcissus* seeds in the light at 10°C compared with 5°C, yet if the seeds at 5°C were then transferred to 10°C (i.e. 20-5-10°C), no additional germination occurred (Fig. 5B). A drop in temperature after a warm period (i.e. autumn following summer) was necessary for germination; germination at the same temperature did not, however, take place after a cold period (i.e. spring following winter).

Effect of temperature on shoot formation. Shoots started to develop 28 – 56 d after seed germination in both species (Fig. 8). In *N. pseudonarcissus*, shoot formation occurred more rapidly at the same temperature as germination (10°C) compared with the cooler temperature of 5°C. The opposite occurred in *G. nivalis*: shoot formation started later and proceeded more slowly at the same temperature as germination (15°C), taking just under 300 d to complete, compared with shoot formation at 10°C, which finished by 134 d (Fig. 8).

DISCUSSION

Temperature, light, desiccation and seed maturity all affected seed dormancy and germination in *G. nivalis* and *N. pseudonarcissus*. Simulating natural seasonal patterns over long periods enabled germination in the majority of seeds to be achieved, eventually. This investigation provides the first comprehensive long-duration investigation of the relatively complex environmental and temporal regulation of seed germination in these species. The responses to these factors make ecological sense in the context of the type of dormancy exhibited by these species' seeds, whilst the new information will also aid the conservation of these species both *ex-situ* and *in-situ*.

According to the classification system of Baskin and Baskin (2001, 2004), seeds of these species may be said to possess morphophysiological dormancy (Newton *et al.*, 2013). Primary dormancy appears to be imposed comparatively late in seed development when capsules change from green to yellow (Fig. 7). Seeds are shed immature from yellow and brown capsules that dehisce at the end of spring (Newton *et al.*, 2013). Seeds of *G. nivalis* possess an oil-rich appendage or elaiosome which is attractive to ants; ants collect and carry seeds back to the nest where the elaiosome is eaten by the colony and the seed discarded unharmed (Handel & Beattie, 1990; Budnikov & Kricsfalusy, 1994). In contrast, seeds of *N. pseudonarcissus* are dispersed passively close to the parent plant, often rolling into cracks in the soil or under vegetation (Newton, 2011). After dispersal, embryos elongate within seeds during warm summer temperatures (Newton *et al*., 2013). Germination occurred outdoors in early autumn following a drop in temperature (Fig. 1) and was greatest in the laboratory at 15°C in *G. nivalis* (Figs 5C and 7) and 10°C in *N. pseudonarcissus* (Figs 3 and 5).

Germination requirements are thus similar to *N. bulbicodium* (Thompson, 1977), a Belgian population of *N. pseudonarcissus* (Vandelook & van Assche, 2008) and *N. hispanicus* (Copete *et al.*, 2011), all of which require warm summer temperatures for germination in the autumn. Warm summer temperatures do not only affect seed germination, but are also necessary for dormancy release in bulbs of *N. tazetta* L. var. *chinensis* M.Roem. (Li *et al.*, 2012).

Shoots formed at temperatures which promoted substantial germination (10°C in *N. pseudonarcissus* and 15°C in *G. nivalis*) and also at temperatures 5°C cooler, concurring with results of Vandelook and van Assche (2008) for *N. pseudonarcissus*; however, with a 5°C drop in temperature after germination, shoots developed more rapidly (at 10 *cf.* 15°C) in *G. nivalis* but more slowly (at 5 *cf.* 10°C) in *N. pseudonarcissus* (Fig. 8). Slower development of shoots at cold temperatures in *N. pseudonarcissus* may delay emergence until spring, minimising the risk of frost damage (Mondoni *et al.*, 2008; Vandelook & van Assche, 2008), while the more rapid development of *G. nivalis* shoots over winter may give this species a competitive advantage in early spring when competition for light, nutrients and water would be less (Fenner & Thompson, 2005).

Light reduced seed germination significantly in both species, but particularly in *G. nivalis*. Similar responses of greater germination in darkness than in light have been reported in *N. hispanicus* (Copete *et al.*, 2011), *N. longispathus* (Herranz *et al.*, 2013a) and *N. alcaracensis* (Herranz *et al.*, 2013b), and to a lesser extent in *N. serotinus* and *N. cavanillesii* (Marquis & Draper, 2012). Inhibition of seed germination can occur at very low levels of light; for example, in *Chenopodium album* L. and *Galium aparine* L. inhibition occurs at light levels >

70 µmol m^{-2} s^{-1} and in *Amaranthus caudatus* L. above 5 µmol m^{-2} s^{-1} (Bliss & Smith, 1985). Light levels in laboratory incubators (15 – 50 µmol m^{-2} s^{-1}) were roughly comparable to minimum light levels on the soil surface of dappled shady woodland or light levels under grass on a sunny day (Table 2). These light levels were sufficient to virtually prevent germination in *G. nivalis* (Fig. 5C) and reduce germination and spread it over successive seasons in *N. pseudonarcissus* (Fig. 4 and 5B), in contrast to Caldwell and Wallace (1955) who suggested *N. pseudonarcissus* seeds were insensitive to light. The response to light in these species matched the post-dispersal ecological microsites of their seeds very well: seeds of *G. nivalis* in ants' nests and tunnels would be in darkness, while limited light would reach *N. pseudonarcissus* seeds in dispersal microsites which would decrease over the summer as seeds are increasingly shaded by surrounding vegetation.

The presence or absence of light during the summer period had little influence on subsequent germination in the autumn. This suggests that light is not involved in dormancy alleviation. Subsequently, however, light inhibits, or darkness promotes, germination in the autumn period. Seeds of *G. nivalis* showed a greater sensitivity to light, as a brief exposure to light (when assessing germination regularly) reduced germination slightly in *G. nivalis* but not in *N. pseudonarcissus* (Fig. 5A).

Germination at alternating temperatures was significantly reduced compared with equivalent mean constant temperatures in both species; similar responses of greater germination at constant temperatures have been reported in the amaryllis *Hippeastrum* × *hydridum* Hort. (Carpenter & Ostmark, 1988) and *N. hispanicus* (Copete *et al.*, 2011). The mean difference between the maximum and minimum temperature at the study site over a year was 8.8 ±

0.21°C in air but only 2.8 ± 0.09°C just below the soil surface. Buried *G. nivalis* seeds would thus experience only small diurnal temperature fluctuations while *N. pseudonarcissus* seeds, likely buried under thick vegetation at the end of summer, would probably be exposed to fluctuations less than those recorded in air but greater than those for *G. nivalis*. Reduced germination at alternating temperatures in *G. nivalis* and *N. pseudonarcissus* compared with equivalent constant temperatures (Fig. 4) may be due to an inhibitory effect of alternating temperatures on germination; however, a more likely explanation is that the amplitude was wider than the narrow range of constant temperatures at which germination was maximal, e.g. as shown in several *Orobanche* L. species (Kebreab & Murdoch, 1999).

Light and alternating temperatures function as depth-sensing mechanisms to ensure that small seeds (< 2 mg) germinate close to the soil surface so that seedlings are able to emerge (Fenner & Thompson, 2005). Seeds of *N. pseudonarcissus* and *G. nivalis* weigh 7 – 25 mg at dispersal (Newton *et al.*, 2013); as larger seeds they would emerge from greater depths and consequently this would not be a driver to evolve a requirement for alternating temperatures and light for germination (Murdoch, 1983; Pons, 1992). Sensitivity to alternating temperatures, and especially light, may instead be an adaptation to prevent germination on or near the soil surface, reducing the probability of seedling death due to drying (Pons, 1992; Thanos, Georghiou & Delipetrou, 1994; Fenner & Thompson, 2005).

Seeds of *N. pseudonarcissus* survived drying at 15% RH [compared with 75% RH in the field (Newton *et al.*, 2013)] at different stages and also repeatedly throughout the warm summer period (Fig. 6A, B). Dormancy of *N. pseudonarcissus* seeds in the first autumn increased when seeds were dried and even more so when seeds were dried later in the summer period.

Viability after desiccation in the summer period in *N. pseudonarcissus*, however, was only slightly reduced, and seed germination during the second autumn resulted in similar final germination in all treatments. Germination in *G. nivalis* followed a similar trend to that in *N. pseudonarcissus* of reduced germination in the first autumn following drying during the summer period (Fig. 6C). Germination in *G. nivalis* was low (< 50%) and quite variable: seeds were sensitive to fungal infection which was exacerbated by desiccation, and is likely to have negatively affected viability and consequently germination. Fungal infection usually originated on the elaiosome and rapidly spread over the whole seed. Elaiosome removal by ants once buried may reduce fungal growth, as the nutrient-rich contents of the elaiosome are consumed (Handel & Beattie, 1990); mechanical removal of the elaiosome did not, however, result in improved seed survival and germination (Newton, 2011). Burial of *G. nivalis* seeds beneath the soil surface by ants presumably prevents seeds drying and thus reduces the risk of dehydration and death; in contrast, seeds of *N. pseudonarcissus*, which are likely to be more exposed and consequently vulnerable to drying on the soil surface during summer, were able to withstand several periods of desiccation with little effect (< 15% decline) on viability.

As the duration in the warm summer period increased, so did seed germination in the autumn, with 120 d at 20°C required for maximal germination in *N. pseudonarcissus* and 152 d in *G. nivalis* (Fig. 2), supporting germination patterns obtained for a Belgian population of *N. pseudonarcissus* (Vandelook & van Assche, 2008). Similar patterns of greater dormancy release with increased duration of incubation at dormancy breaking temperatures have been reported for *Anemone ranunculoides* L. (Mondoni *et al.*, 2009b) and *N. hispanicus* (Copete *et al.*, 2011), in contrast to other temperate geophytes, *Hyacanthoides non-scripta, Scilla bifolia* (Vandelook & van Assche, 2008) and *A. nemorosa* (Mondoni *et al.*, 2008), which require only a short (approximately 30 d) warm stratification for maximum seed germination at

cooler autumn temperatures. Although Karssen, Derkx and Post (1988) demonstrated in *Spergula arvensis* L. that a condensed annual temperature cycle relieved and induced dormancy in a similar manner to that observed in the field, this was not the case in *N. pseudonarcissus* and *G. nivalis*, as reducing seasonal duration reduced germination (Fig. 3). The requirement for such a long warm period prior to placing *N. pseudonarcissus* and *G. nivalis* seeds into germination conditions highlights the dangers of reducing stratification periods in order to obtain more rapid germination in laboratory tests.

Results suggest that primary dormancy is alleviated gradually during the warm summer period, allowing germination at cooler autumn temperatures (Fig. 2). If, however, suitable germination temperatures are absent [e.g. when *N. pseudonarcissus* seeds were transferred directly from 20°C to 5°C (Fig. 5B)], cold winter-like temperatures induced secondary dormancy, thereby preventing germination when these seeds were moved to suitable germination temperatures [in this case, 10°C (spring)]. Induction of secondary dormancy by cold temperatures has been observed in *Papaver rhoeas* L. (Baskin *et al*., 2002) and *N. hispanicus* (Copete *et al.*, 2011). Germination was also prevented in a proportion of seeds by light (Fig. 5B) and deeper dormancy (Figs 6, 7). Seeds which did not germinate in the first season favourable for seedling establishment (autumn) typically persisted until the second, and in some cases, even the third autumn, when germination occurred (Figs 2-7). Dormancy cycling thus occurs in both *G. nivalis* and *N. pseudonarcissus*, with dormancy removal in subsequent warm summer periods in viable seeds that did not germinate in the first autumn, resulting in germination in the second and even third autumn periods.

In summary, dormancy is induced relatively late in development in *G. nivalis* and *N. pseudonarcissus* seeds. Seeds are shed immature in spring into a moist environment and dispersed underground by ants (*G. nivalis*) or onto the soil surface into cracks and under vegetation (*N. pseudonarcissus*). It is unlikely that *G. nivalis* seeds would be exposed to desiccation below the soil surface in moist habitats. Seeds of *N. pseudonarcissus* would, however, be more vulnerable to drying, and are able to withstand drying events during the summer period. Over the warm summer period embryos elongate in both species, primary dormancy is gradually alleviated and germination occurs with a temperature drop in the autumn, first in *G. nivalis* and shortly thereafter in *N. pseudonarcissus*. There is no arrest in seed development during summer following shedding, nor is there a halt in shoot development following germination; subtle changes in temperature control embryo growth, seed germination and seedling development. An arrest in this process only occurs when temperatures are not suitable (too warm or too cool) or conditions (e.g. light) prevent germination. In such situations where germination does not occur, secondary dormancy may be induced (e.g. by cold winter temperatures). Requirements for darkness and constant temperatures would be met for *G. nivalis* when seeds are underground and it is unlikely that viable seeds in these safe sites would not germinate in the first autumn. This may also be true for *N. pseudonarcissus*, although light, alternating temperatures and deeper dormancy (due to drying, for example) may function to delay germination. It is therefore unlikely that large quantities of seed of either of these species would persist in the soil seed bank beyond the first autumn. However, the small proportion of seeds that may not germinate in the first autumn would most likely re-enter dormancy at cold winter temperatures, preventing germination in spring, and would survive to germinate in the second, or possibly even third, autumn.

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FIGURE CAPTIONS

Figure 1. Radicle emergence, shoot formation and shoot emergence (± 95% binomial confidence intervals) of (A) *Narcissus pseudonarcissus* and (B) *Galanthus nivalis* seeds set to germinate outdoors and (C) mean maximum, minimum and weighted fortnightly temperature (± s.e.) at Wakehurst Place in West Sussex, UK. Data at each sampling time in (A) and (B) represents an independent sample. Tick marks indicate the 1st of each month.

Figure 2. Effect of duration of the first summer period on cumulative germination of *Narcissus pseudonarcissus* and *Galanthus nivalis* seeds incubated at the temperatures (T) shown (summer, autumn, winter, spring) in light (L, 8/16 h light/dark photoperiod) or darkness (D). The first summer varied in duration from 0 to 98 d (A) or 0 to 168 d (B and C); thereafter all seasons were 84 d in duration. A "year" consisted of four seasonal periods (1 = the first year, 2 = the second year, and 3 = the third year).. Probit analysis was applied to the first autumn germination data (shown by fitted lines). The 140 d treatment in *G. nivalis* (C) was excluded from the analysis, as seed viability (and thus germination) was negatively affected by high levels of fungal contamination. The 95% binomial confidence intervals are shown for final total germination.

Figure 3. Effect of varying the duration of all seasons on cumulative germination of *Narcissus pseudonarcissus* and *Galanthus nivalis* seeds incubated at the temperatures (T) shown (summer, autumn, winter, spring) and in light (L, 8/16 h light/dark photoperiod) or darkness (D). A "year" consisted of four seasonal periods (1 = the first year, 2 = the second year, 3 = the third year, 4 = the fourth year, and 5 = the fifth year). Tests with a seasonal duration of 28, 56 or 84 d were finished at the end of the fifth, third, or second autumn, respectively.

Figure 4. Effect of constant (CN) or alternating temperatures with 10°C amplitude (A10, 12/12 h) or 15°C amplitude (A15, 8/16 h, with 8 h at the warmer temperature) on cumulative germination of *Narcissus pseudonarcissus* and *Galanthus nivalis* seeds incubated at the temperatures (T) shown (summer, autumn, winter, spring) in light (L) or darkness (D). Alternating temperatures provided the same daily arithmetic mean as the equivalent constant value. A "year" consisted of four seasonal periods (1 = the first year, 2 = the second year, and 3 = the third year).

Figure 5. (A) Effect of light (L) or dark (D) on cumulative germination of *Narcissus pseudonarcissus* and *Galanthus nivalis* seeds incubated at the temperatures (T) shown (summer, autumn, winter, spring). Light (8/16 h photoperiod) was provided throughout (LLLL), in all seasons except summer (DLLL), only in summer (LDDD), or not at all (DDDD). For 2008 seed lots in which light was excluded, germination was assessed at weekly intervals either under laboratory light (DDDD-L) or a safe, green light (DDDD-D). (B) Effect of light and different seasonal temperatures on cumulative seed germination of *N. pseudonarcissus*. (C) Effect of light and different seasonal temperatures on cumulative seed germination of *G. nivalis*. A "year" consisted of four seasonal periods (1 = the first year, 2 = the second year, and 3 = the third year).

Figure 6. Effect of drying *Narcissus pseudonarcissus* and *Galanthus nivalis* seeds once, after 0, 28, 56, 84, 91, or 105 d moist incubation, or two (after each of 42 and 84 d), three (24, 56, 84 d) or four times (21, 42, 63, 84 d) during the first summer period on cumulative germination. Germination temperatures (T) are shown (summer, autumn, winter, spring) and seeds were incubated in the light (L, 8/16 h light/dark photoperiod) or dark (D). The control

was not dried (ND). All treatments received an imbibed 84 d summer and 84 d autumn period. A "year" consisted of four seasonal periods (1 = the first year, 2 = the second year, and 3 = the third year). Probit analysis was applied to the first autumn germination data (shown by fitted lines).

Figure 7. Effect of seed maturity on cumulative germination in the dark (D) of *Galanthus nivalis* seeds extracted from green (G) or yellow (Y) capsules incubated at the temperatures (T) shown. A "year" consisted of four seasonal periods (1 = the first year, 2 = the second year, and 3 = the third year).

Figure 8. Cumulative shoot formation (± 95% binomial confidence intervals) in germinated seeds of *Narcissus pseudonarcissus* and *Galanthus nivalis* at the same constant temperature as germination (triangles: 10°C for *N. pseudonarcissus* and 15°C for *G. nivalis*) or a constant 5°C lower than the germination temperature (squares: 5°C for *N. pseudonarcissus* and 10°C for *G. nivalis*) in the light (8/16 h light/dark photoperiod). Seedlings possessed a minimum radicle length of 5 mm when transferred to experimental conditions (day 0).

Table 1. Dates of collection of seed lots of *Narcissus pseudonarcissus* from Loder Valley Nature Reserve (Loder; Ldr in figures) and Forest Edge Farm near Ringwood (Ringwood; Rwd in figures) and *Galanthus nivalis* from Wakehurst Place (Wakehurst), and the experiments in which they were used. For ease of reference, figure numbers correspond to experiment numbers.

No.	Experiment	Species	Seed lot(s)	Collection date
1	Outdoor experiment	*N. pseudonarcissus*	Loder 2011	17 May 2011
		G. nivalis	Wakehurst 2011	18 May 2011
2	Varied duration of first summer period experiment	*N. pseudonarcissus*	Loder 2006	7 Jun 2006
			Ringwood 2007	2 June 2007
		G. nivalis	Wakehurst 2008	14 – 28 May 2008
3	Varied seasonal duration experiment	*N. pseudonarcissus*	Ringwood 2006	9 June 2006
			Loder 2008	30 May 2008
			Ringwood 2008	1 June 2008
		G. nivalis	Wakehurst 2008	14 – 28 May 2008
4	Constant and alternating temperature experiment	*N. pseudonarcissus*	Loder 2007	31 May 2007
			Loder 2008	30 May 2008
		G. nivalis	Wakehurst 2008	14 – 28 May 2008
5	Light and darkness experiment	*N. pseudonarcissus*	Loder 2007	31 May 2007
			Loder 2008	30 May 2008
			Ringwood 2008	1 June 2008
		G. nivalis	Wakehurst 2007	17 May 2007
			Wakehurst 2008	14 – 28 May 2008
6	Drying experiment	*N. pseudonarcissus*	Ringwood 2006	9 June 2006
			Ringwood 2008	1 June 2008
		G. nivalis	Wakehurst 2008	14 – 28 May 2008
7	Seed maturity experiment	*G. nivalis*	Wakehurst 2007	17 May 2007
8	Cumulative shoot formation experiment	*N. pseudonarcissus*	Loder 2009	28 May 2009
		G. nivalis	Wakehurst 2009	29 May 2009

Table 2. Photon flux density range measured on bright and cloudy days in four microsites in the Loder Valley Nature Reserve and in incubators in which seed germination experiments were conducted.

Microsite	Photon flux density on a bright day (µmol m^{-2} s^{-1})	Photon flux density on a cloudy day (µmol m^{-2} s^{-1})
Bare ground (unshaded)	1000 – 2000	50 – 400
Woodland (dappled shade)	20 – 200	4 – 8
Grass cover (shaded)	10 – 50	0.3 – 6.0
Leaf litter cover (deep shade)	0.4 – 4.0	0.1 – 1.0
Laboratory incubators	15 – 50 µmol m^{-2} s^{-1}	

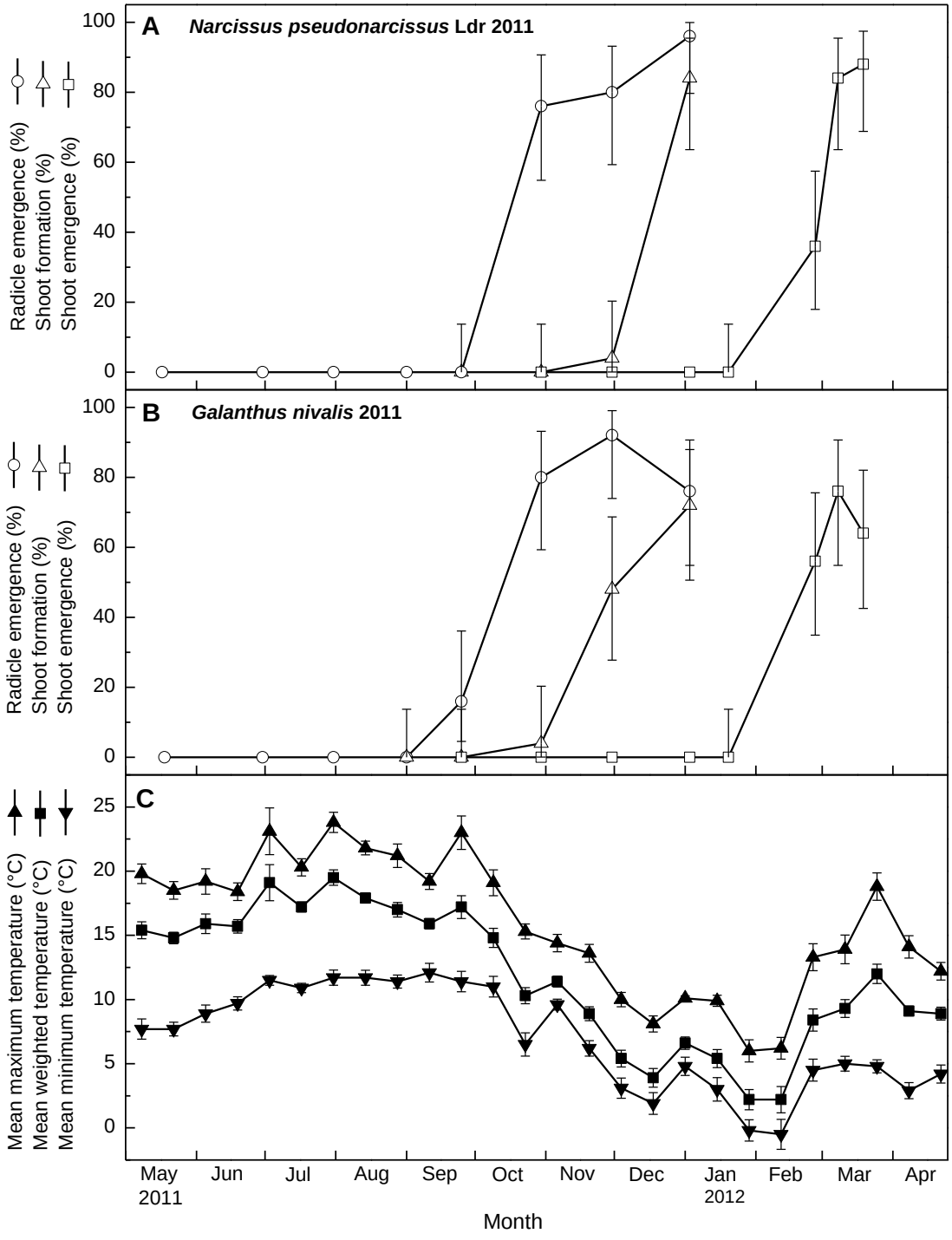
A
Narcissus pseudonarcissus Ldr 2011
B
Galanthus nivalis 2011
C
Radicle emergence (%)
Shoot formation (%)
Shoot emergence (%)
Mean maximum temperature (°C)
Mean weighted temperature (°C)
Mean minimum temperature (°C)
0
20
40
60
80
100
0
5
10
15
20
25
May
2011
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2012
Feb
Mar
Apr
Month

Figure 1

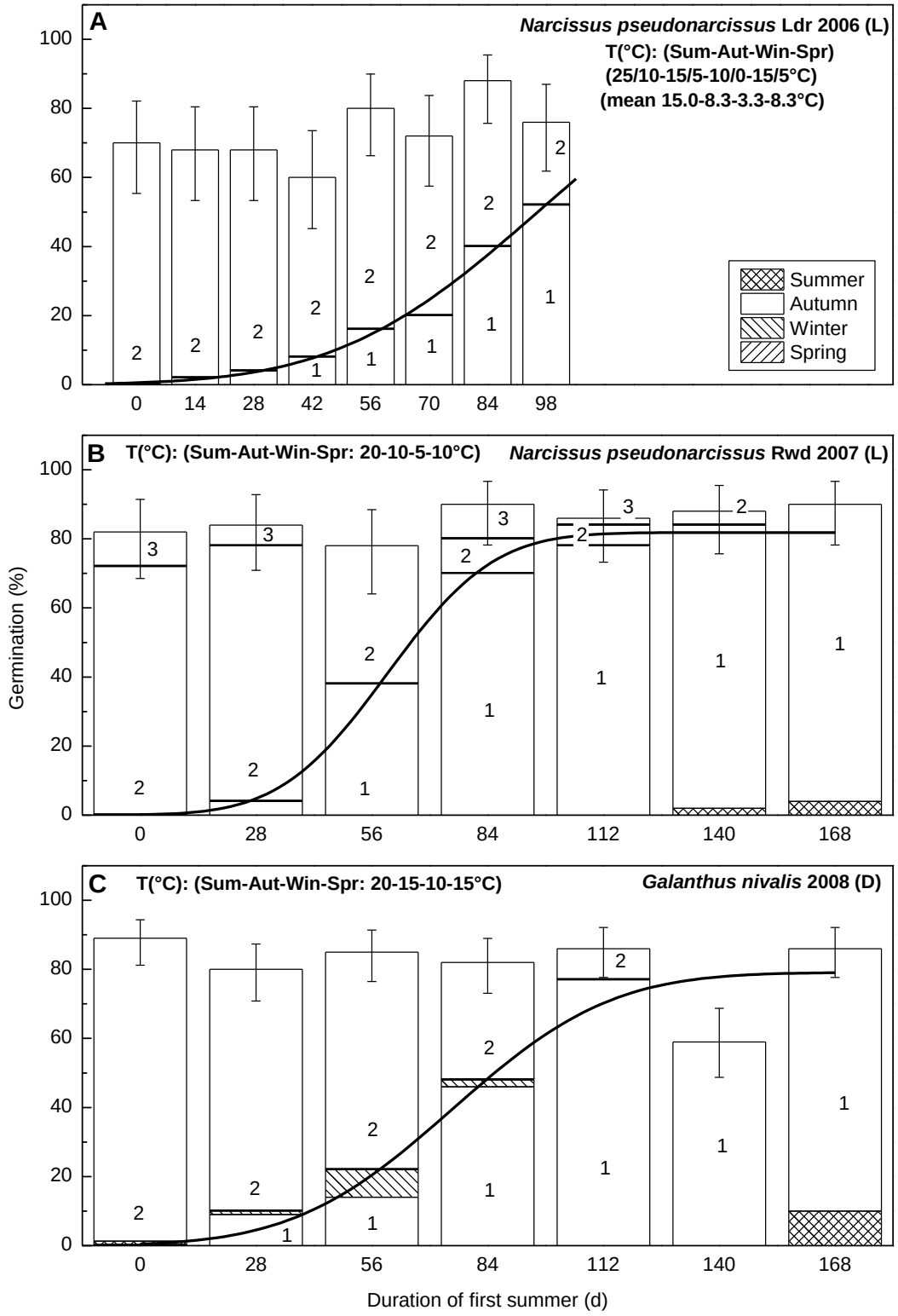
A
Narcissus pseudonarcissus Ldr 2006 (L)
T(°C): (Sum-Aut-Win-Spr)
(25/10-15/5-10/0-15/5°C)
(mean 15.0-8.3-3.3-8.3°C)
Summer
Autumn
Winter
Spring
B
T(°C): (Sum-Aut-Win-Spr: 20-10-5-10°C)
Narcissus pseudonarcissus Rwd 2007 (L)
C
T(°C): (Sum-Aut-Win-Spr: 20-15-10-15°C)
Galanthus nivalis 2008 (D)
Germination (%)
Duration of first summer (d)

Figure 2

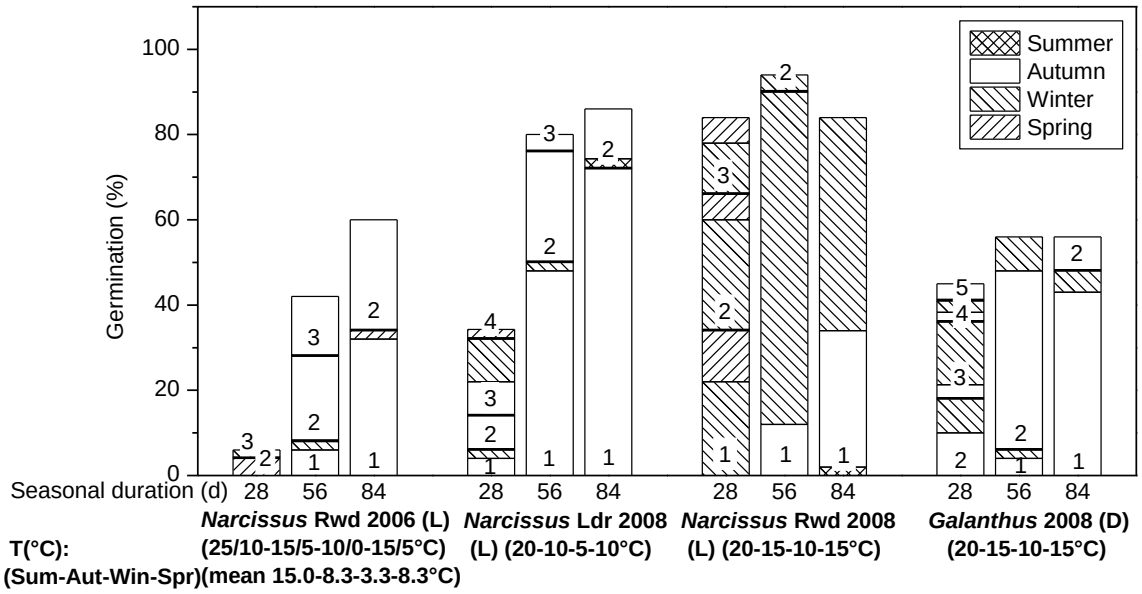
Summer
Autumn
Winter
Spring
Germination (%)
0
20
40
60
80
100
Seasonal duration (d) 28 56 84 28 56 84 28 56 84 28 56 84
Narcissus Rwd 2006 (L) (25/10-15/5-10/0-15/5°C) (mean 15.0-8.3-3.3-8.3°C)
Narcissus Ldr 2008 (L) (20-10-5-10°C)
Narcissus Rwd 2008 (L) (20-15-10-15°C)
Galanthus 2008 (D) (20-15-10-15°C)
T(°C): (Sum-Aut-Win-Spr)

Figure 3

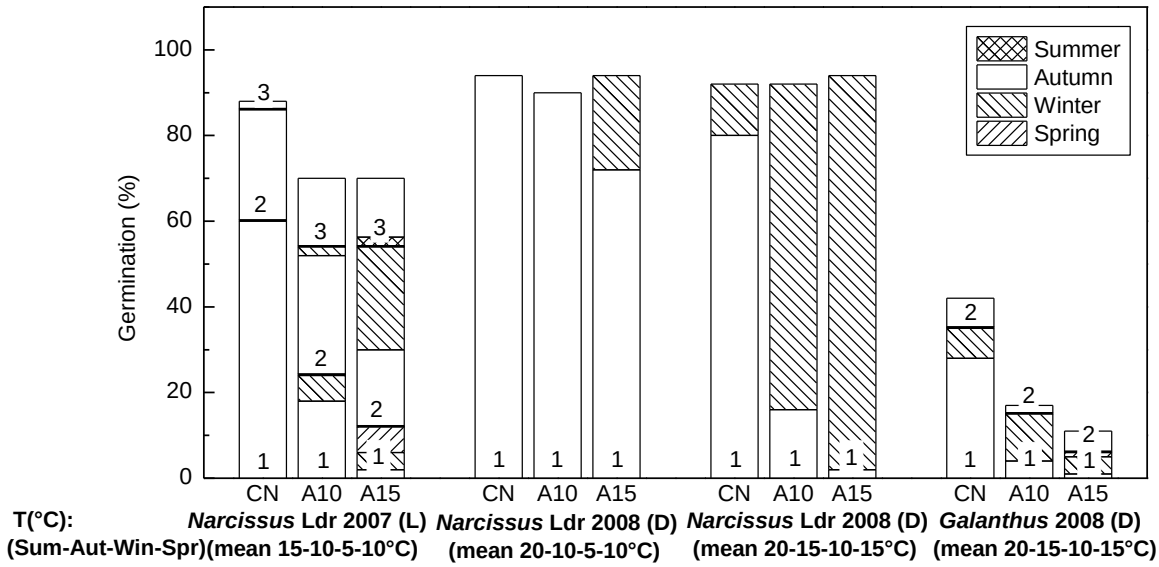
Germination (%)
100
80
60
40
20
0
Summer
Autumn
Winter
Spring
CN A10 A15 CN A10 A15 CN A10 A15 CN A10 A15
T(°C):
(Sum-Aut-Win-Spr)
Narcissus Ldr 2007 (L)
(mean 15-10-5-10°C)
Narcissus Ldr 2008 (D)
(mean 20-10-5-10°C)
Narcissus Ldr 2008 (D)
(mean 20-15-10-15°C)
Galanthus 2008 (D)
(mean 20-15-10-15°C)

Figure 4

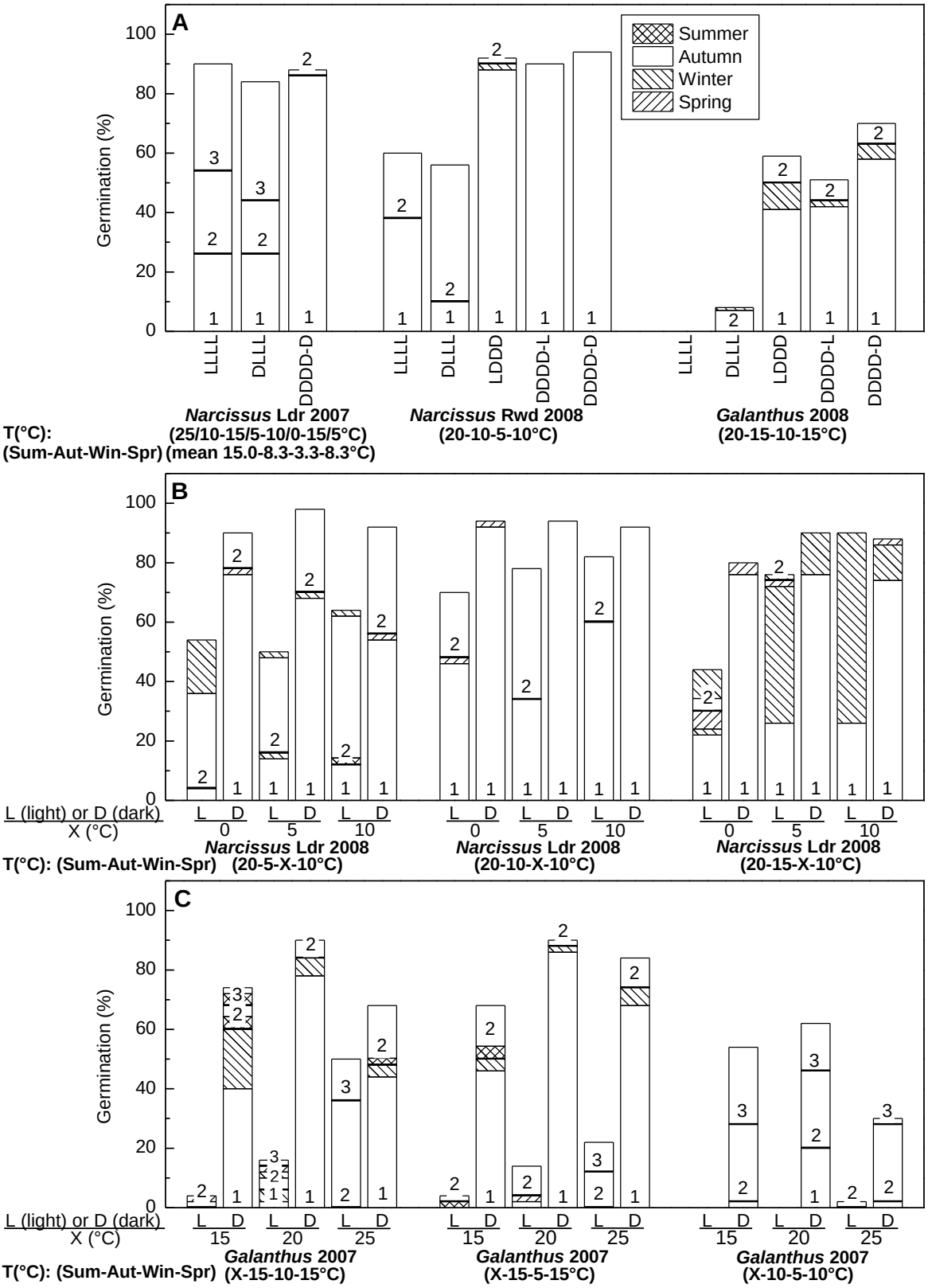
A
Summer
Autumn
Winter
Spring
Germination (%)
LLLL
DLLL
DDDD-D
LLLL
DLLL
LDDD
DDDD-L
DDDD-D
LLLL
DLLL
LDDD
DDDD-L
DDDD-D
Narcissus Ldr 2007
(25/10-15/5-10/0-15/5°C)
Narcissus Rwd 2008
(20-10-5-10°C)
Galanthus 2008
(20-15-10-15°C)
T(°C):
(Sum-Aut-Win-Spr)
(mean 15.0-8.3-3.3-8.3°C)
B
Germination (%)
L (light) or D (dark)
X (°C)
Narcissus Ldr 2008
(20-5-X-10°C)
Narcissus Ldr 2008
(20-10-X-10°C)
Narcissus Ldr 2008
(20-15-X-10°C)
T(°C): (Sum-Aut-Win-Spr)
C
Germination (%)
L (light) or D (dark)
X (°C)
Galanthus 2007
(X-15-10-15°C)
Galanthus 2007
(X-15-5-15°C)
Galanthus 2007
(X-10-5-10°C)
T(°C): (Sum-Aut-Win-Spr)

Figure 5

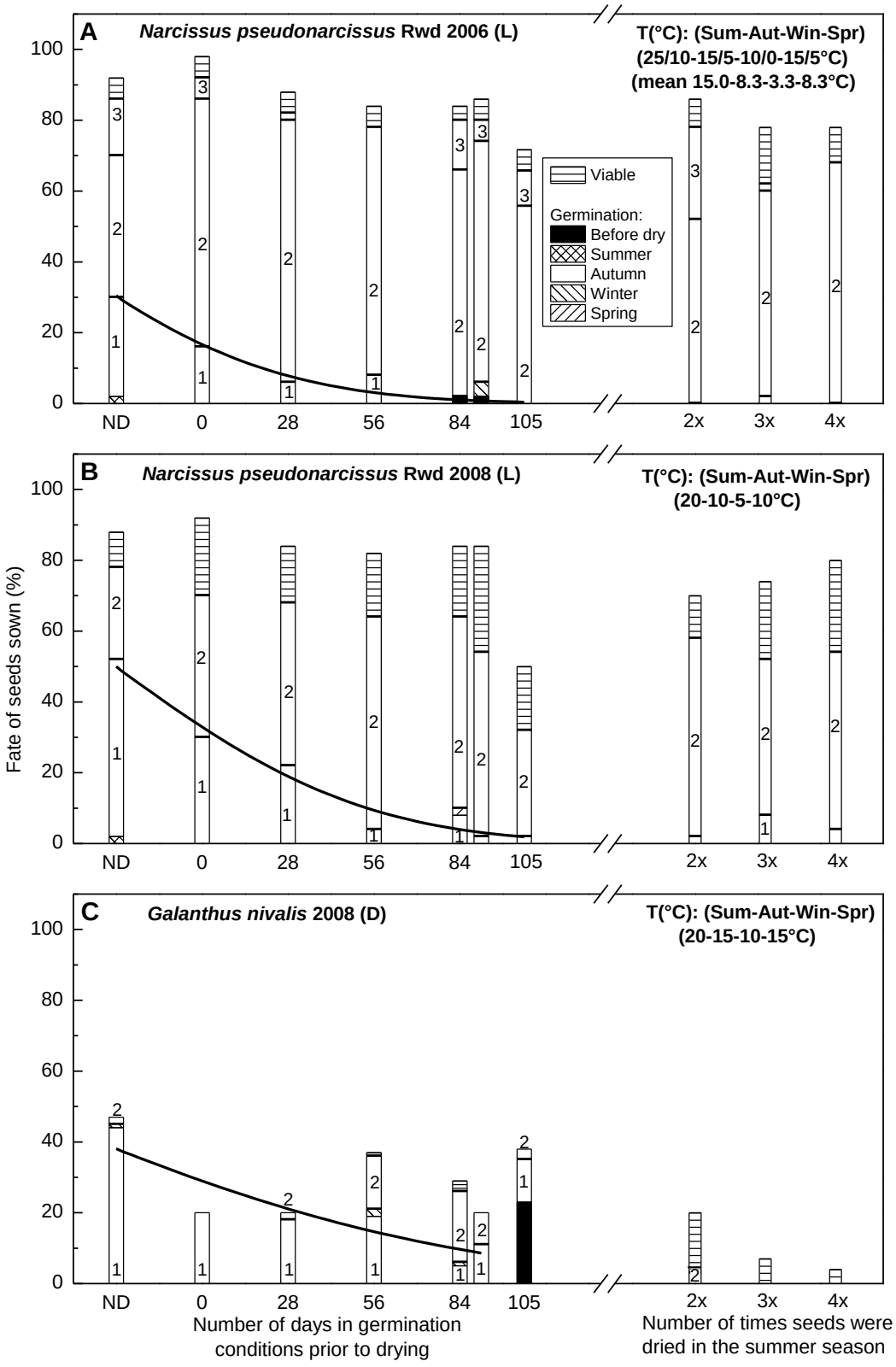
A
Narcissus pseudonarcissus Rwd 2006 (L)
T(°C): (Sum-Aut-Win-Spr)
(25/10-15/5-10/0-15/5°C)
(mean 15.0-8.3-3.3-8.3°C)
Viable
Germination:
Before dry
Summer
Autumn
Winter
Spring
B
Narcissus pseudonarcissus Rwd 2008 (L)
T(°C): (Sum-Aut-Win-Spr)
(20-10-5-10°C)
C
Galanthus nivalis 2008 (D)
T(°C): (Sum-Aut-Win-Spr)
(20-15-10-15°C)
Fate of seeds sown (%)
ND
0
28
56
84
105
2x
3x
4x
Number of days in germination conditions prior to drying
Number of times seeds were dried in the summer season

Figure 6

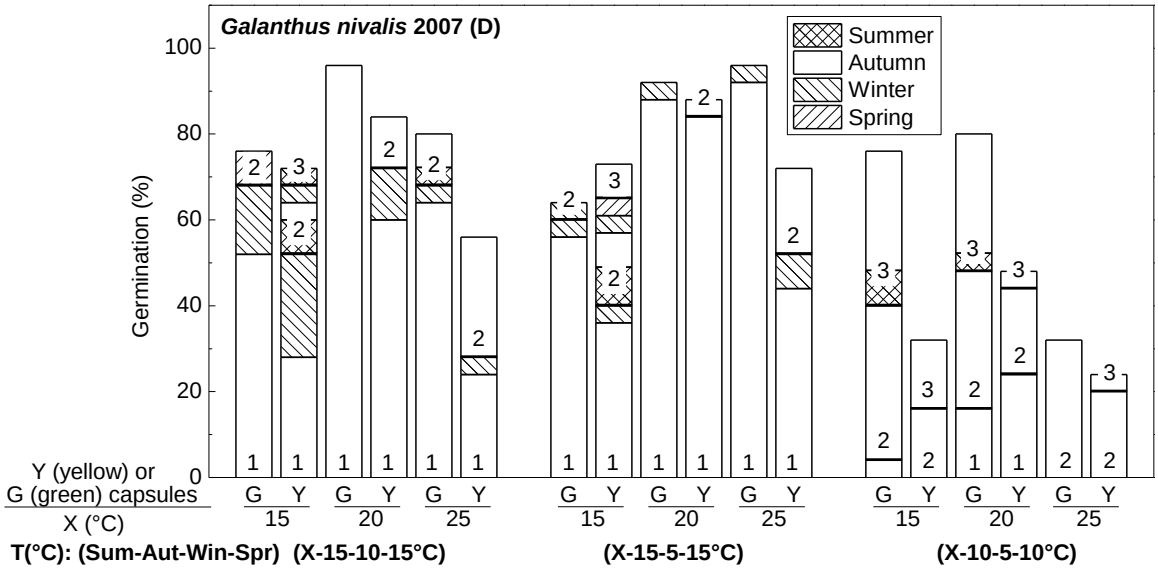
Galanthus nivalis 2007 (D)
Summer
Autumn
Winter
Spring
Germination (%)
Y (yellow) or
G (green) capsules
X (°C)
T(°C): (Sum-Aut-Win-Spr) (X-15-10-15°C)
(X-15-5-15°C)
(X-10-5-10°C)

Figure 7

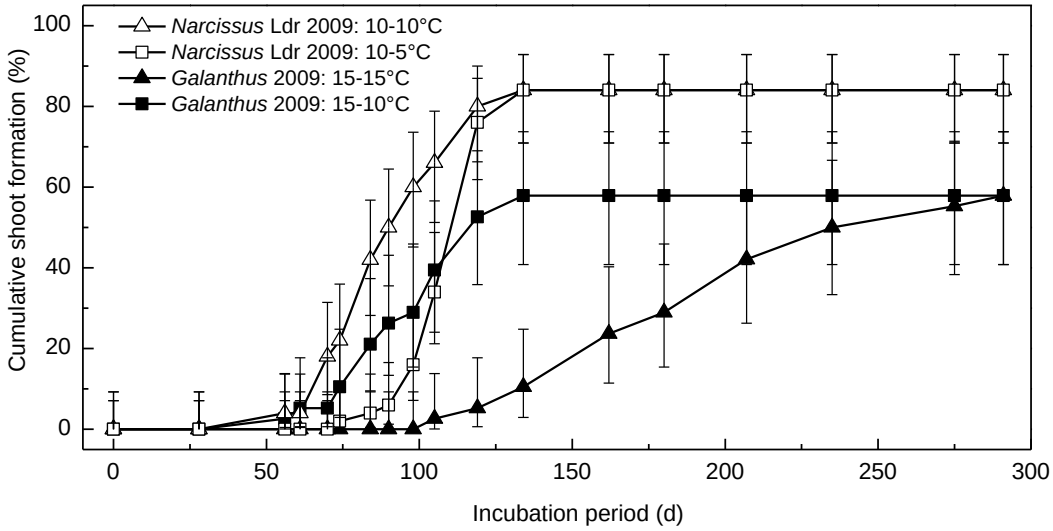
Narcissus Ldr 2009: 10-10°C
Narcissus Ldr 2009: 10-5°C
Galanthus 2009: 15-15°C
Galanthus 2009: 15-10°C
Cumulative shoot formation (%)
Incubation period (d)
0
20
40
60
80
100
0
50
100
150
200
250
300

Figure 8